

Amendments to the Claims

The following listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims

What is claimed is:

1. (Currently amended) A nucleic acid sequence comprising:

$P_x-S_x-B_n-(ZR)-Hir-(As_mR)-protein(Y)-T$

where

P_x is a promoter sequence;

S_x is a nucleic acid encoding a signal sequence or leader sequence;

B_n is 1-15 codons, when n is an integer from 1 to 15, or a chemical bond, when $n=0$;

Z is a codon for lysine or arginine;

R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence coding for hirudin or hirudin derivative ~~which is at least 40% homologous to a natural hirudin isoform~~;

As_m is a chemical bond, when $m=0$, or 1-10 codons, when m is an integer from 1 to 10;

$protein(Y)$ is a nucleic acid sequence encoding a protein that is produced in and secreted by yeast; and

T is an untranslated expression-enhancing nucleic acid sequence.

2. (Original) The nucleic acid of claim 1, wherein $protein(Y)$ encodes for mini-proinsulin or a derivative thereof.

3. (Withdrawn) The nucleic acid of claim 1, wherein $protein(Y)$ encodes for interleukin, lymphokine, or interferon.

4. (Withdrawn) A fusion protein encoded by the nucleic acid of claim 1.

5. (Withdrawn) A fusion protein encoded by the nucleic acid of claim 2.

6. (Withdrawn) A fusion protein encoded by the nucleic acid of claim 3.
7. (Original) A multicopy vector comprising the nucleic acid of claim 1.
8. (Original) A plasmid comprising the nucleic acid of claim 1.
9. (Original) A host cell comprising the nucleic acid of claim 1, as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally.
10. (Original) The host cell of claim 9, wherein the host cell is a yeast.
11. (Original) The host cell of claim 10, wherein the yeast is selected from *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Hansenula polymorpha*, and *Pichia pastoris*.
12. (Original) A host cell comprising the multicopy vector of claim 7.
13. (Original) A host cell comprising the plasmid of claim 8.
14. (Original) A process of fermentative production of fusion protein, comprising:
expressing the nucleic acid of the host cell of claim 9 to form the fusion protein in a fermentation supernatant of a cell culture; and
isolating the fusion protein from the fermentation supernatant of the cell culture.
15. (Original) The process of claim 14, wherein isolating the fusion protein comprises adjusting the pH of the fermentation supernatant to about 2.5 to 3.5 to precipitate non-desired proteins and to form a precipitation supernatant, and isolating the fusion protein from the precipitation supernatant.
16. (Currently amended) The process of claim 15, further comprising separating, prior to adjusting the pH of the fermentation supernatant, the fermentation supernatant from the host cell, and after separating the fermentation supernatant

from the host cell, the host cell is repeatedly cultured in fresh medium to form additional supernatant from each culture, and fusion protein is isolated from each additional supernatant.

17. (Original) The process of claim 14, wherein:

isolating the fusion protein comprises precipitating the fusion protein from the fermentation supernatant, and

the method further comprises removing the protein encoded by protein(Y) from the fusion protein, and concentrating the protein encoded by protein(Y) by at least one of microfiltration, hydrophobic interaction chromatography, and ion exchange chromatography.

18. (Original) A process of fermentative production of fusion protein, comprising:

expressing the nucleic acid of the host cell of claim 12 to form the fusion protein in a supernatant of a cell culture; and

isolating the fusion protein from the supernatant of the cell culture.

19. (Original) A process of fermentative production of fusion protein, comprising:

expressing the nucleic acid of the host cell of claim 13 to form the fusion protein in a supernatant of a cell culture; and

isolating the fusion protein from the supernatant of the cell culture.

20. (Currently amended) A process for preparing insulin, comprising:

expressing and isolating a fusion protein by the process of claim 14, wherein protein(Y) encodes for mini-proinsulin or a derivative thereof;

releasing insulin into a reaction mixture by treating the fusion protein with trypsin and carboxypeptidase B; and

isolating the insulin from the reaction mixture.